ISTITUTO ITALIANO DI TECNOLOGIA

5TH-7TH October 2025

RNA-based innovative therapies for neurological disorders

HOTEL EXCELSIOR, Via Partenope, 48 Napoli 80121 Italia

National Center for Gene Therapy and Drugs based on RNA Technology

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Italiadomani PIANO NAZIONALE DI RIPRESA E RESILIENZA

RNA &GENETHERAPY

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Scientific Committee

Stefano Gustincich

Angelo Antonini

Elena Bonora

Salvatore Oliviero

Giuseppe Pignataro

Angelo Poletti

Maurizio Taglialatela

Logistic Committee

Alice Agliano

Eva Ferri

DOUBLE EM SRL

Schedule

Day 1 – Sunday 5th of October

Welcome ***16.30-17:30***

16.30 – 17.00 Registration

17.00 - 17.15 Welcome Dr. Gustincich Stefano - IIT

17.15 - 17.30 Welcome Prof. Rizzuto Rosario - CN3, UniPD

Session 1 – RNA Metabolism and therapeutics – Plenary Lecture ***17.30 -18.30***

Chairs Dr. Gustincich -IIT and Prof. Oliviero - UniTO

17.30 - 18.30 Invited Speaker

Prof. Adrian R. Krainer
St. Giles Foundation Professor
Cancer Center Program Co-Leader
Cold Spring Harbor Laboratory USA

Antisense Modulation of mRNA Splicing for CNS Disorders

Poster Session 1 – Abstracts #1-27 (Sala Colonna) ***18.30 – 19.30***

Aperitivo Buffet Dinner (Sala Colonna) ***from 19.30***

Day 2 – Monday 6th of October

Session 1 – RNA Metabolism and therapeutics ***9.00 – 10.40***

Chairs Dr. Gustincich - IIT and Prof. Oliviero S. - UniTO

9.00 - 9.20 Dr. Gustincich Stefano - IIT

SINEUPs as a new therapeutic platform for haploinsufficiencies and complex diseases of the nervous system

9.20 - 9.40 Prof. Tartaglia Gian Gaetano – IIT

Breaking Boundaries in Biotechnology: Designing RNA molecules for Diagnostics and Therapeutics

Schedule

9.40 - 10.00 Dr. Cancedda Laura – IIT

NKCC1 miRNA Gene Therapy as a Potential Treatment for Normal Pressure Hydrocephalus

10.00 - 10.20 Prof. Oliviero Salvatore – UniTO

Non-coding RNAs in Brain Developmental Diseases

10.20 - 10.40 Dr. De Pietri Tonelli Davide – IIT

piRNA Pathway Functions in the Aging Brain

Coffee Break ***10.40 -11.10***

Session 2 – Neurodev ***11.10 – 12.40***

Chairs Prof. Bonora – UniBO and Cancedda - IIT

11.10 -11.30 Prof. Presutti Carlo- UniROMA1

Use of small non coding RNAs to target RNA molecules in neurodevelopmental disorders

11.30 – 11.50 Dr. Barberis Andrea – IIT

SINEUPs for the Treatment of Genetic Epilepsies Linked to Impaired GABAergic Inhibition

11.50 - 12.10 Dr. Espinoza Stefano – UniPO

SINEUP Targeting PRPF31 for Retinitis Pigmentosa 11: a Proof of Concept for an Innovative, Effective Therapy

12.10 - 12.40 Invited Speaker

Prof. Gaia Novarino
Executive Vice President
Institute of Science and Technology Austria (ISTA), Wien, Austria

From Molecular Programs to Neural Systems: Navigating the Complex Landscape of Autism Spectrum

Poster Session 2 and Buffet Lunch – Abstracts #28-43 (Sala Colonna) ***12.40 – 13.40***

Schedule

Session 3 – AD/PD/ALS ***13.45 – 16.15***

Chairs Prof. Antonini – UniPD and Prof. Frigeri - UniBA

13.45 - 14.05 Prof. Rosa Alessandro - UniROMA1

The RNA-binding Protein ELAVL4/HuD: from Basic Molecular Mechanisms to a New Possible Target in ALS

14.05 - 14.25 Prof. Ballarino Monica - UniROMA1

Long Noncoding RNAs as a Therapeutic Toolkit for Neuromuscular Disorders

14.25 - 14.45 Prof. Bozzoni Irene - UniROMA1

Targeting m6A RNA modification for the treatment of ALS

14.45 - 15.05 Prof. Denti Michela – UniTN

Therapeutic Modulation of RNA Splicing for the Treatment of 4R Tauopathies

15.05 - 15.25 Prof. Pizzo Paola - UniPD

Targeting the extracellular ATP/P2X7 receptor signaling axis to fight neuroinflammation in Alzheimer's disease

15.25 -15.45 Prof. Antonini Angelo- UniPD

Clinical, Biological and Genetic Characterization of the PADUA-CESNE Cohort

15.45 - 16.15 Invited Speaker

Prof. Piero Fratta
Professor of Cellular and Molecular Neuroscience
Department of Neuromuscular Diseases, UCL, London, UK

Novel RNA gene therapy approaches for ALS

Coffee Break ***16.15 – 16.45***

Schedule

Session 4 – Expanded repeats ***16.45 – 19.00***

Chairs Prof. Poletti -UniMI and Prof. Provenzani - UniTN

16.45 - 17.05 Prof. Poletti Angelo – UniMI

Targeting Expanded Repeat-Derived Toxic Proteins: Emerging Interventions for Motor Neuron Disorders

17.05 - 17.25 Prof. Corti Stefania– UniMI

Therapeutic Targeting of C9ORF72-G4C2 Repeats: An Innovative Approach to Mitigate Neurotoxicity in ALS/FTD

17.25 - 17.45 Prof. Biagioli Marta – UniTN

The Conserved *circHTT(2,3,4,5,6)* in Huntington's Disease: From Pathological Insight to Therapeutic Innovation

17.45 - 18.05 Prof. Martello Graziano- UniPD

Testing the Therapeutic Potential of MTF1 Gene Delivery in Human *in vitro* Models of Huntington's Disease

18.05 - 18.25 Dr. Maglione Vittorio - NEUROMED

Nanoparticle-mediated Delivery of Therapeutic RNA in Pre-clinical Models of Neurodegenerative Diseases

18.30 – 19.00 Invited Speaker

Prof. Davide Trotti
Dept. of Neuroscience, Thomas Jefferson University, USA
Director of Research, Weinberg ALS Center
Vickie and Jack Farber Institute for Neuroscience

Genome Instability and Innate Immune Stress Response in Neurodegeneration

Schedule

Day 3 – Tuesday 7th of October

Session 5 – Stroke and Retina ***9.00 – 10.40***

Chairs Prof. Taglialatela – UniNA and Prof. Pignataro – UniNA

9.00 - 9.20 Prof. Pignataro Giuseppe- UniNA

MicroRNAs as mediators of endogenous mechanisms involved in brain adaptation to ischemic insult

9.20 - 9.40 Dr. Siciliano Velia – IIT

RNA-based Bioengineering Approaches to Therapy

9.40 - 10.00 Prof. Surace Enrico Maria- UniNA

Transcriptional Repression as a Disease-modifying Gene Therapy for Gangliosidosis

10.00 - 10.20 Prof. Siciliano Gabriele – UniPI

Retina-Gut-Brain Axis and Paths to Interfere at RNA level with Neurodegeneration in ALS and AD: from Mouse Models to Clinical Correlates

10.20 - 10.40 Prof. De Falco Sandro– CNR

Proof-of-concept of Gene Editing Approach to Inhibit Pathological Neovascularization in Age-related Macular Degeneration

Coffee Break ***10.40 – 11.10***

11.10 – 12.10 Plenary Lecture

Prof. Michael Chopp
Vice Chairman for Research of the Department of Neurology
Scientific Director of the Henry Ford Neuroscience Institute
The Zoltan J. Kovacs Chair in Neuroscience Research, Detroit, USA

Small Extracellular Vesicles-outsized Therapeutic Effects on Stroke and Neural Injury

Concluding remarks ***12.10 – 12.30***

Invited Speakers

Prof. Adrian R. Krainer

St. Giles Foundation Professor

Cancer Center Program Co-Leader

Cold Spring Harbor Laboratory, New York (USA)

Antisense Oligonucleotide Therapies for Neurological Disorders

In collaboration with Ionis Pharmaceuticals and Biogen, we previously developed nusinersen (Spinraza), an antisense oligonucleotide (ASO) that modulates alternative splicing of *SMN2* exon 7, restoring normal levels of functional SMN protein in the context of spinal muscular atrophy (SMA). The long duration of action of CNS-administered ASOs like Spinraza allows infrequent dosing by lumbar puncture, providing a feasible and effective approach to treat neurological disorders. Consequently, many ASOs are being developed against relevant targets in neurology and neuro-oncology. Splice-switching ASOs, in particular, are highly versatile, because of the pervasiveness of pre-mRNA splicing. They can be designed to upregulate or downregulate the expression of target genes, to change the relative levels of alternatively spliced isoforms, to correct defective splicing, or to bypass frame-disrupting mutations. I will describe several applications of this powerful technology.

Short Bio

Adrian Krainer, Ph.D. is the St. Giles Foundation Professor at Cold Spring Harbor Laboratory. His lab studies the mechanisms and regulation of messenger RNA splicing in human cells, and the role of splicing dysfunction in genetic diseases and cancer. His lab is also engaged in the preclinical development of antisense-oligonucleotide drugs that target RNA splicing or other RNA-processing steps. Together with Ionis Pharmaceuticals and Biogen, Adrian's lab developed nusinersen (Spinraza), which was approved by the FDA in 2016 as the first treatment for spinal muscular atrophy, a neurodegenerative disease that was the leading genetic cause of infant mortality. He is a member of the U.S. National Academy of Sciences, the National Academy of Medicine, and the American Academy of Arts & Sciences, and has received multiple major scientific awards. Adrian served as President of the RNA Society in 2014, and currently serves on the advisory boards of several scientific centers and networks, non-profit foundations, and biotechnology companies in the U.S., Europe, and Latin America. He is a co-founder and Director of Stoke Therapeutics.

Invited Speakers

Prof. Novarino Gaia

Executive Vice President, Institute of Science and Technology Austria (ISTA)

Vienna, Austria

From cell to systems: Navigating the Complex Landscape of Autism Spectrum Disorders

Autism spectrum disorders (ASD) are a heterogeneous group of disorders rooted in genetic bases. Although genetic studies have identified many ASD-risk genes over the past few years, it remains essentially unknown whether mutations in different genes result in similar molecular and cellular states within the brain. Over the past years, we have generated a large cohort of ASD mouse models carrying mutations in top, high-risk ASD genes. The cohort includes 11 mouse lines and span chromatin remodelers, ubiquitin-related genes, metabolically relevant genes, and synaptic proteins. Employing these validated mouse models, we performed simultaneous single nucleus RNA and ATAC sequencing across brain regions, developmental stages, and sexes, totaling over 300 samples. We achieved this by pioneering an approach that allowed us to sequence RNA and DNA from the same nucleus on a large scale. With this unique dataset, we could address precise questions regarding convergence and divergences across different ASD models, time windows affected, and sex differences.

Short Bio

Gaia Novarino, an Italian native, received her PhD degree in Developmental Biology from the University of Rome La Sapienza (Italy). During her PhD, she moved to Germany to join the Max Delbruck Center for Molecular Medicine, where she focused on studying chloride transporters associated with human genetic disorders. In 2010, she moved to San Diego to do her postdoc at the School of Medicine of the University of California San Diego, where she studied the genetics of neurodevelopmental disorders. In 2014, she joined the Institute of Science and Technology Austria (ISTA), first as an Assistant Professor and then as Full Professor. She is an ERC grant holder (ERCstg and ERCcons) and a Simons Foundation Investigator. In 2016, Gaia won the prestigious Boehringer Ingelheim FENS Research Award for her research on molecular mechanisms underlying human neurodevelopmental disorders. Since 2021, she has been the Vice President of Science Education and, since 2024, the Executive Vice President at ISTA Austria. Gaia's research interest lies in the understanding of the molecular mechanisms underlying neurodevelopmental and neuropsychiatric disorders.

Invited Speakers

Prof. Fratta Pietro

Professor of Cellular and Molecular Neuroscience

University College London and The Francis Crick Institute, London, (UK)

Novel RNA gene therapy approaches for ALS

TDP-43 mislocalisation is a core feature of ALS and other neurodegenerative diseases. The nuclear loss gives rise to numerous alterations of RNA processing, including cryptic splicing and polyadenylation. We have previously described and characterised specific mis-splicing events that play a role in disease pathogenesis, how these can be corrected to rescue neuronal phenotypes, and also how the splicing dysfunction can be used to regulate the expression of potential gene therapies. We here expand on the above by providing further insight in the mechanisms and consequences of TDP-43 loss.

Short Bio

Pietro Fratta is Professor of Cellular and Molecular Neuroscience at the UCL Queen Square Institute of Neurology and the Francis Crick Institute. He previously worked at King's College London, University of Southern California and San Raffaele Scientific Research Institute.

His laboratory combines patient tissue, iPSCs and mouse models to understand molecular disease mechanisms underlying ALS and neurodegeneration. Over the last five years, his laboratory has developed a novel gene therapy approach to limit therapeutic expression to diseased cells, uncovered the role of UNC13A in ALS and developed a splice-switching ASO therapeutics now brought forward by Trace Neuroscience, and identified novel biomarkers for ALS.

He is also a Consultant Neurologist at the National Hospital for Neurology and Neurosurgery in London, where he established National Kennedy's Disease clinic and an MND Genetics clinic.

Invited Speakers

Prof. Trotti Davide

Professor, Dept. of Neuroscience, Thomas Jefferson University

Director of Research, Weinberg ALS Center
Vickie and Jack Farber Institute for Neuroscience

Pennsylvania (USA)

Transposable Elements as Immune Triggers in Neurodegeneration

This presentation explores how transposable element (TE) mobilization contributes to neurodegenerative processes in Alzheimer's disease (AD) and C9-ALS/FTD. It highlights a c-Jun-dependent mechanism that opens up previously repressed regions of the genome, allowing human endogenous retroviruses (ERVs) to generate RNA-DNA hybrids. These hybrids can activate the cGAS-STING pathway, triggering an innate immune response that may drive neurodegeneration. The research shows that these effects can impair neurogenesis—particularly in the hippocampus of AD models—and extends similar findings to C9-ALS/FTD. Notably, the work also demonstrates that retro-transcriptase inhibitors (such as lamivudine) can reduce the buildup of RNA-DNA hybrids and help restore a healthier cellular environment. Together, these insights point toward a shared, TE-driven pathway of neuroinflammation in multiple neurodegenerative disorders and highlight potential therapeutic angles to mitigate disease progression.

Short Bio

Dr. Davide Trotti is a tenured Professor of Neuroscience at Thomas Jefferson University and Research Director of the Weinberg ALS Center in Philadelphia. He earned his B.S. and M.S. degrees from the Universita' degli Studi in Milan, Italy, before completing a Ph.D. in Neuroscience with training at the University of Oslo and postdoctoral work at Harvard Medical School. Dr. Davide Trotti is internationally recognized for his research on amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). His work dissects the cellular and molecular mechanisms of neurodegeneration, with a particular focus on C9orf72-associated toxic pathways, transposable element activation, protein aggregation, and the role of innate immune signaling. He is also pioneering innovative therapeutic strategies, including the development of CAR T cells directed against microglia as a novel approach for neurodegenerative disease. Dr. Trotti's research program has been continuously supported by major NIH grants and private foundations. He serves on NIH study sections, Department of Defense review panels, and scientific advisory boards, reflecting his leadership and sustained contributions to the neurodegeneration field.

Invited Speakers

Prof. Chopp Michael

Vice Chairman for Research of the Department of Neurology
Scientific Director of the Henry Ford Neuroscience Institute
Education & Research Building, 2799 West Grand Boulevard, Detroit, MI (USA)

Small extracellular vesicles-outsized therapeutic effects on stroke and neural injury

Small extracellular vesicles play a pivotal role in intercellular communication. Here, I will describe some of our work, primarily on small extracellular vesicles derived from healthy cerebral endothelial cells (CEC-sEVs) and demonstrate how these CEC-sEVs may be used to reverse vascular damage and the prothrombotic, procoagulant , and proinflammatory state of vascular endothelial cells post stroke and neural injury (actually induced by "toxic" clot/damaged endothelial cell EVs). and thereby greatly promote neurological recovery. Means to enhance selective brain delivery and therapeutic efficacy of CEC-sEVs for stroke and other neurological diseases will be presented. In addition, I will describe our studies on how stroke and neural injury alter the microbiota composition and the diversity of EVs derived from the gut, and how these EVs adversely impact neurological and behavioral function, and I will describe ways to treat these gut EV mediated neurological and behavioral dysfunctions. sEVs by regulating intercellular communication and recipient cell function may be harnessed as potent therapeutics for stroke, neurodegenerative and other diseases.

Short Bio

Dr. Michael Chopp is Division Head for Research Department of Neurology and Zolton J Kovacs Chair in Neuroscience Research at Henry Ford Health, He is also a Distinguished Professor of Physics at Oakland University, and a Professor of Physiology at Michigan State University. Dr. Chopp received his PhD in Physics from New York University. His research is primarily focused on neurovascular restorative and protective therapies for cerebrovascular disease and injury. His scientific achievements, include pioneering work using pharmacological and cell-based and extracellular vesicle-based therapies for stroke, traumatic brain injury, peripheral neuropathy and neurodegenerative diseases. He has >780 peer reviewed publications (h-index 166) and > 50 book chapters. His numerous awards include: Top 10 Contributions to Medicine (2001), AHA-Thomas Willis Award (2015), WSO Lecture of Excellence (2012), and Barbro B. Johansson Award (2016)

Poster Abstract – Session 1

P01

Targeted Increase of CHD2 Protein in Neurological Disorders: The SINEUP Strategy

Alvino Filomena Grazia1, Alice Tata1, Lorenzo Milesi1, Brigitta Bonaldo2, Stefano Espinoza3,4, Carlotta Bon3, Stefano Gustincich3, Marta Biagioli1

1. NeuroEpigenetics Laboratory, Department of Cellular, Computational and Integrative Biology, University of Trento, 38123 Trento, Italy. 2. Department of Bioscience, University of Milano La Statale, 20122 Milano, Italy. 3. Center for Human Technologies, Non-coding RNAs and RNA-based Therapeutics, Istituto Italiano di Tecnologia (IIT), 16152 Genova, Italy; 4. Department of Health Sciences and Research Center on Autoimmune and Allergic Diseases (CAAD), University of Piemonte Orientale (UPO), 28100 Novara, Italy.

CHD2 is a chromatin remodeling factor whose haploinsufficiency, caused by loss-of-function mutations, is linked to epilepsy, intellectual disability, and autism spectrum disorder (ASD). No targeted therapies currently exist. We propose an RNA-based therapeutic approach using SINEUPs, long non-coding antisense RNAs that selectively enhance translation of the target mRNA, restoring protein levels to physiological ranges. SINEUPs combine a Binding Domain (BD) for specificity and an Effector Domain (ED) that increases translation. We designed 30 constructs with 10 BDs targeting unstructured regions of CHD2 mRNA and 3 EDs (SINEB2, IRES, FRAM). Screening in HEK293T cells, which endogenously express CHD2, revealed that SINEUPs targeting less structured regions significantly enhanced CHD2 protein levels. We generated CRISPR/Cas9-edited iPSC lines with heterozygous CHD2 frameshift mutations: four 4 bp and one 21 bp deletion clones. In these models, SINEUPs targeting internal regions of CHD2 mRNA effectively restored protein expression. Building on our approach validated for CHD8, another ASD-related gene, we are expanding the testing of SINEUP molecules to the murine gene Chd2, aiming at injecting AAV9-SINEUP-Chd2 into Chd2+/- mouse model to explore the ability of this tool to revert motor, social, cognitive dysfunctions and transcriptomic alterations. These preliminary findings support the therapeutic potential of SINEUPs for the treatment of CHD2 haploinsufficiency.

Poster Abstract – Session 1

P02

Modeling patient phenotypes using patient-specific brain organoids

Sonia Amato

Neurodegenerative diseases (NDs) are a group of heterogeneous disorders marked by the progressive loss of specific neuronal populations, resulting in functional decline of the central nervous system. This group includes Parkinson's disease (PD), Alzheimer's disease (AD), Huntington's disease (HD), spinocerebellar ataxias, and spinal and bulbar muscular atrophy. While genetic predisposition is a major factor, aging and environmental influences also contribute to disease onset and progression. A shared hallmark among many NDs is the accumulation of misfolded proteins—for instance, amyloid-β and tau in AD, α-synuclein in PD, and mutant huntingtin in HD. Despite growing insight into their molecular and cellular basis, effective disease-modifying therapies remain elusive.

Human induced pluripotent stem cells (h-iPSCs), reprogrammed from somatic cells, preserve the donor's genetic makeup and can differentiate into various neural cell types. When used to generate brain organoids—three-dimensional cultures that mimic key aspects of human brain architecture—they offer a powerful in vitro system to model NDs.

This study demonstrates that patient-derived brain organoids can reproduce disease-relevant phenotypes, including hallmark features such as the selective degeneration of dopaminergic neurons in PD. These findings underscore the potential of 3D brain organoids as physiologically relevant platforms for investigating pathogenic processes and therapeutic strategies in NDs.

Poster Abstract – Session 1

P03

Design and synthesis of small molecules as modulators of microRNA function

Andreozzi G., Pignataro G., Sparaco R., Severino B., Caliendo, Santagada, and Fiorino F.

Andreozzi, Sparaco, Severino, Caliendo, Santagada, Fiorino= Dipartimento di Farmacia, Università degli Studi di Napoli "Federico II", Via D. Montesano, 49-80131 Napoli, Italy. Pignataro=Division of Pharmacology, Department of Neuroscience, School of Medicine, University of Naples Federico II, Naples, Italy

MicroRNAs are critically involved in various diseases. Recent studies indicate that miRNAs can be targeted by molecules, indeed, in this study a series of small molecules were synthesized as potential miRNA ligands. The design results from the combination of structural elements capable of interfering with the functions of certain miRNAs. The focus of the study was mainly on miR-150-5p and miR-181b-5p which are involved in hypoxia, ischaemia and stroke. The structures of them were predicted using the ROSIE FarFar2 web server and Molecular Docking studies were performed with AutoDock Vina. Subsequently, the Prediction of Activity Spectra for Substances program was used to predict the biological activities of the compounds. In addition, a toxicity test was conducted, and all the synthesised compounds were tested by reproducing a real ischaemic stroke condition under OGD, oxygen and glucose deprivation. The compounds were found to be not cytotoxic and cell viability generally remained low, except for three compounds, which increased cell viability at the lowest concentrations. Then, the compounds were evaluated for their ability to modulate miRNA expression which generally remained unchanged under normal conditions, expect for two of them, where treatment restored levels to those of the control group. This finding allows investigating the potential of these molecules as therapeutic targets and this will allow further refinement of research and progress towards in vivo studies.

Poster Abstract – Session 1

P04

Biodegradable bicompartmental microneedles as a platform for mRNA-LNP vaccination

Roberta Arpino1, Elena Lagreca1, Roberta Passariello2, Atefeh Malek Katahbi1, Daniela Orefice1, Emilia Cioffi1, Stefano Persano3, Giorgia Imparato1, Raffaele Vecchione1 and Paolo Antonio Netti1,2

1 Center for Advanced Biomaterials for Health Care (CABHC), Istituto Italiano di Tecnologia, Largo Barsanti e Matteucci 53, 80125 Naples, Italy. 2 Interdisciplinary Research Centre on Biomaterials (CRIB), Università degli Studi di Napoli Federico II, P. le Tecchio 80, 80125 Naples, Italy. 3Dipartimento di Oncologia ed Emato-Oncologia, Università degli Studi di Milano, Milan, Italy.

The application of biodegradable microneedles (MNs) for intradermal vaccination is rapidly advancing. MNs improve patient compliance by offering a painless alternative to conventional needles and can enhance immune responses with lower vaccine doses by targeting skin antigen-presenting cells. This is particularly promising for mRNA-based therapies including anticancer vaccines by exploiting lipid nanoparticles (LNPs) to stabilize the mRNA. Based on this, we developed a “Matryoshka-like” MNs system loading LNPs encapsulating either EGFP-mRNA or OVA-mRNA. First, we validated implantation performance and GFP expression in a 3D human dermal model for 10 days post-administration by confocal microscopy, histology, and cryosectioning. To assess preliminary immune activation, we performed flow cytometry with a conjugated antibody specific to the OVA-derived peptide SIINFEKL bound to H-2Kb of MHC class I, a tool widely used to follow antigen presentation, evaluate T-cell responses, and support mRNA-based anticancer therapies which may involve several tumors including brain tumor. This approach improves stability, delivery efficiency, and immune activation, highlighting the potential of this novel platform for next-generation immunotherapeutic agents in personalized medicine.

Poster Abstract – Session 1

P05

RNA-based therapeutics for neurodevelopmental disorders: focus on STX1B haploinsufficiency

J.S. Martinheira Da Silva1, L. Menta1, A. Riva1, G. Balagura1, C. Bon4, S. Gustincich4, P. Striano1,3, F. Zara1,2, S. Cappato2, R. Bocciardi1,2.

1Department of Neurosciences, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Sciences (DINOGMI), University of Genoa, Genoa, Italy 2Medical Genetics Unit, IRCCS Istituto Giannina Gaslini, Genoa, Italy 3Neurology and Muscular Diseases Unit, IRCCS Istituto Giannina Gaslini, Genoa, Italy 4Center for Human Technologies, Non-coding RNAs and RNA-based therapeutics, Italian Institute of Technology (IIT), 16152 Genoa, Italy.

"STX1B (OMIM#601485) is a key protein in the SNARE complex, crucial for synaptic vesicle fusion and neurotransmitters release. STX1B gene variants causing haploinsufficiency, are linked to a severe, early-onset neurodevelopmental disorder (NDD) known as Generalized epilepsy with febrile seizures plus, type 9 (OMIM#616172).

Our aim is to investigate RNA-based technologies to enhance STX1B expression at both transcriptional and post-transcriptional levels, and rescue haploinsufficiency.

To this purpose we analyzed the STX1B mRNA stability and structure, and we applied CRISPRa and SINEUPs based approaches, to increase gene expression.

We found that STX1B mRNA stability varies with cell type. CRISPRa significantly increased STX1B mRNA, although the protein remained undetectable by Western Blot. Additionally, we identified sequences on the STX1B mRNA using in-silico tools and used them to design and synthesize SINEUPs. We are currently doing the screening of the SINEUPs targeting STX1B mRNA.

In summary, we are investigating RNA-based therapies for neurodevelopmental disorders caused by STX1B haploinsufficiency. While our CRISPRa approach successfully increased STX1B mRNA, it didn't raise protein levels. This suggests potential issues with mRNA stability, translation, or protein degradation. We are now working on a combined strategy of transcriptional and post-transcriptional methods to restore STX1B protein.

Poster Abstract – Session 1

P06

SINEUP Technology: Multi-Target RNA for Dopaminergic Neuroprotection in Parkinson's Disease

Bon C.1, Shabalova A.1, Nava L.1, Novello S.4, Burton C. L.1, Gomes G. M.1, Bugelli C.3, Vaccari C.3, Fogli M.3, Ros G.1, Peruzzo O.1, Persichetti F.2, Santoro C2, Oliviero S.4, Greggio E.4, Bubacco L.4, Tonini R.1, Espinoza S.1, 2 and Gustincich S.1

1 Istituto Italiano di Tecnologia, Genova, Italy; 2 Dipartimento di Scienze della Salute, Università del Piemonte Orientale, Novara, Italy; 3 Dipartimento di Scienze della Vita e Biologia dei Sistemi, Università di Torino, Italy; 4Dipartimento di Biologia, Università di Padova, Italy.

SINEUP is a class of antisense long non-coding RNAs that enhance mRNA translation. Synthetic SINEUPs can be custom-designed to target nearly any gene, offering therapeutic promise. In previous work, we showed that a SINEUP targeting Glial Cell-Derived Neurotrophic Factor (GDNF) increased its endogenous expression by 80%, improving motor function and dopaminergic (DA) neuron survival in a Parkinson's disease (PD) mouse model without side effects. For broader neuroprotection, modulation of multiple molecular targets may be required. c-RET, GDNF's receptor, and glucocerebrosidase (GBA) are key contributors to PD pathology. c-RET potentiates GDNF's therapeutic effects, while enhancing GBA expression reduces α-synuclein aggregation and neuronal loss. Mitochondrial dysfunction and lysosomal impairment are additional drivers of neurodegeneration. LAMP2a and NRF2, which support autophagy-lysosomal function and oxidative stress defense, are further promising targets. In this study, we developed two multi-target SINEUP constructs: multiSINEUP-PD.01, upregulating GDNF, c-RET, and GBA; and multiSINEUP-PD.02, enhancing LAMP2a, NRF2, and c-RET. Both were effective in vitro, increasing target expression and reducing α-synuclein aggregation in primary neurons treated with pre-formed fibrils. Preliminary in vivo studies using the 6-OHDA model of PD demonstrated that multiSINEUP-PD.01 effectively prevented DA neuron degeneration in the substantia nigra pars compacta and significantly enhanced

Poster Abstract – Session 1

P07

SAM68 is a crucial post-transcriptional regulator of cardiogenesis

Laura Broglia1,†, Alessandro Dasti1,†, Maria Carla Antonelli1,†, Guy Aoun1, Sabrina D'Agostino2#, Andrea Vandelli1, Alexandros Armaos1, Magdalena Arnal Segura1, Tian V Tian3, Davide Mariani4,5, Alessio Colantoni5, Maria Paola Paronetto6, Stefano Gustincich2, Elias Bechara1* and Gian Gaetano Tartaglia1*

1Center for Human Technologies, RNA Systems Biology, Istituto Italiano di Tecnologia, Genoa, 16152, Italy. 2Center for Human Technologies, Non-coding RNAs and RNA-based therapeutics, Istituto Italiano di Tecnologia, Genoa, 16152, Italy. 3Vall d'Hebron Institute of Oncology, Barcelona, 08035, Spain. 4Center for Human Technologies, Non coding RNAs in Physiology and Pathology, Istituto Italiano di Tecnologia, Via Enrico Melen 83, 16153, Genoa, Italy 5Department of Biology and Biotechnologies "C. Darwin", Sapienza University of Rome, Piazzale Aldo Moro 5, 00185, Rome, Italy 6Department of Movement, Human and Health Sciences, University of Rome "Foro Italico", Rome, Italy † Joint authors. # Current address: RNA Biology and Molecular Therapeutics Team, Centre for Cancer Drug Discovery, Institute of Cancer Research, London, SM2 5NG, UK.

RNA-binding proteins (RBPs) of the STAR family orchestrate mammalian development, yet their precise contributions to lineage specification remain unclear. Here, using CRISPR-Cas9 knockouts and multi-omics approaches we dissect the roles of two STAR proteins, SAM68 and QKI, in mouse embryonic stem cells (mESCs). Both RBPs prove essential for mESC proliferation, self-renewal, and differentiation into beating cardiomyocytes. Strikingly, SAM68 and QKI govern largely distinct post-transcriptional programs. QKI functions early to regulate the expression of cardiac transcription factors and structural genes, whereas SAM68 acts predominantly at later stages of cardiomyocyte differentiation. We uncover a novel role for SAM68 in driving the biogenesis of cardiac-enriched circular RNAs by binding intronic regions flanking back-splice sites and associating with NF90/110. Beyond circRNA production, SAM68 directly engages with the 5′ UTR of Gata4 mRNA—encoding a master cardiogenic transcription factor—and enhances its translation. Together, these findings position SAM68 as a multifunctional post-transcriptional hub that coordinates alternative splicing, circular RNA generation, and translational control to orchestrate cardiac lineage specification. This study broadens our understanding of STAR protein function and highlights the crucial role of SAM68 in shaping post-transcriptional regulatory networks during early development.

Poster Abstract – Session 1

P08

Identification of RNAs interacting with epigenetic factors involved in neurodegeneration

Livia Caizzi, Marco Gaspari, Chiara Cicconetti, Mirko Scrivano, Chiara Bugelli, Claudia Vaccari, Salvatore Oliviero

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Growing evidence highlights the function of RNA molecules as epigenetic regulators. Diverse classes of RNAs, particularly long non-coding RNAs (lncRNAs) have been shown to modulate gene expression by acting as scaffold for proteins or by targeting DNA regions through their secondary structure. Notably, lncRNAs exhibit high cell- and tissue-specificity, and their variety has expanded in mammals in parallel with the evolution of brain complexity, indicating a pivotal role in neural developmental process. In line with this, in neurodegenerative diseases, specific lncRNAs have been found dysregulated, supporting their emerging role as potential therapeutic targets. This project aims to investigate how RNA molecules modulate the binding of epigenetic regulators in human neuronal models with the goal of advancing RNA-based epigenetic therapies. To systematically identify RNAs interacting with epigenetic factors, we optimized a protocol based on single-end enhanced CLIP (seCLIP) that takes the advantage of biotin-streptavidin system to recover high-stringency interacting RNAs. Integration of biotin-CLIP with transcriptomic and epigenomic datasets will reveal convergent RNA networks linked to epigenetic dysfunction. Candidate RNAs will be functionally validated using antisense oligonucleotides (ASOs) in neuronal models and, ideally, in human ESC-derived cerebral organoids. This approach may enable the development of targeted RNA-based interventions for neurodegenerative diseases.

Poster Abstract – Session 1

P09

Correcting G>A point mutations causing fALS by Site-directed RNA editing

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Site-directed RNA editing (SDRE) is a promising RNA therapeutic approach for correcting disease-causing point mutations by exploiting the physiological ADAR-mediated A-to-I(G) RNA editing mechanism, offering a safer and more flexible alternative to genome editing.

Despite its potential, no attempt has been made in the context of ALS, a complex disease characterized by the progressive degeneration of upper and lower motor neurons. About 5-10% of ALS cases have a family history (fALS), and about 40-55% of them are due to pathogenic mutations in genes coding for TARDBP, SOD1, FUS, and C9Orf72. Since no drug is currently available for fALS cases with mutations in the TARDBP locus, we applied SDRE to correct the pathogenic c.892G>A TARDBP variant (p.G298S), driving the on-target recruitment of endogenous ADARs by ad hoc guide RNAs (gRNAs) which mimic natural ADAR structures. Specifically, we designed stable circular gRNAs to express in the HEK293 cells in combination with the TARDBP mutated transcript. After co-transfection of vectors expressing gRNAs and mutated transcript, we achieved a correction rate above 50% with negligible bystander editing (~1% at a single site) and a partial rescue of defective splicing due to the mutated TDP-43 protein. We are still optimizing gRNAs, and we plan to evaluate their capability of correcting the TARDBP G298S mutation in fibroblasts or iPSC-derived motoneurons from ALS patients.

Poster Abstract – Session 1

P10

OxymiRs modulation as gene-independent therapeutic approach in primary mitochondrial diseases

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In primary mitochondrial diseases (PMD) associated with severe neurodegenerative and retinal diseases, hypoxic conditions have been shown to partially rescue the phenotype involving multiple hypoxia-response pathways. In this respect, microRNA-mediated simultaneous modulation of more than one hypoxia-related pathway could be a putative strategy to treat these pathologies. Oxygen-responsive microRNAs (OxymiRs) regulation is a mechanism to rapidly modulate cellular metabolism and allow animals to tolerate and survive in reduced oxygen levels. We focus our attention on conserved OxymiRs, ten neuronal miRNAs regulated in anoxia/hypoxia conditions, and on the identification of novel miRNAs from a deep-sea cephalopod species (P. tetracirrhus) naturally adapted to hypoxic environments. Preliminary data indicate a pro-survival effect for downregulation of two conserved OxymiRs families in an in vitro model of PMD. In parallel, among the 92 miRNAs identified in P. tetracirrhus eye we selected a subgroup of 18 novel miRNAs not conserved in humans. Gene Ontology enrichment analysis on predicted targets allowed to identify five miRNAs to have the potential to modulate mitochondrial dysfunction in PMD and are now tested in human in vitro model of PMD. Our work identified conserved and novel species-specific OxymiRs with functional relevance to mitochondrial function in the hypoxia-adapted organisms and will address their possible prospective as therapeutics in PMD.

Poster Abstract – Session 1

P11

Alternative Androgen Receptor Isoform A (AR-A) Mitigates ARpolyQ Toxicity in SBMA

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Spinal and bulbar muscular atrophy (SBMA) is a neurodegenerative disease caused by a CAG repeat expansion in the androgen receptor (AR) gene. This mutation leads to the translation of an elongated polyglutamine (polyQ) tract, and the receptor becomes toxic upon testosterone activation. Since current therapeutic approaches primarily focus on androgen modulation, leading to severe endocrine side effects, new therapeutic approaches have become relevant to counteract the pathology. Different start codons (AUGs) are involved in AR translation. An internal AUG is located downstream of the CAG repeat, leading to the translation of a shorter isoform (AR-A), devoid of the polyQ tract that should not aggregate and should exhibit at least a partial androgenic transcriptional activity. AR.A resulted to be predominantly expressed in the CNS. Moreover, functional characterization demonstrates that AR-A retains partial androgenic activity but lacks aggregation propensity. Subsequently, we evaluated the effect of ARpolyQ and AR-A co-expression, showing its pro-solubilizing effect on ARpolyQ aggregates. To validate these findings in vivo, we utilized a Drosophila melanogaster model of SBMA. Data obtained demonstrated the absence of eye degeneration in AR-A expressing flies, which is instead present in ARpolyQ expressing flies. Moreover, a reduction of eye degeneration has been observed in flies expressing both ARpolyQ and AR-A, corroborating our hypothesis of the AR-A pro-solubilizing effect.

Poster Abstract – Session 1

P12

Anti NKCC1 microRNA gene therapy rescues core symptoms in a mouse model of Hydrocephalus

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Hydrocephalus is a serious medical condition characterized by excess buildup of cerebrospinal fluid (CSF) inside the brain. Currently, the only way to treat hydrocephalus is through surgery to drain the excess fluid, while no effective medications are available to treat the condition. From a molecular standpoint, one of the key players in CSF production under physiological conditions is the sodium-potassium-chloride cotransporter 1 (NKCC1), which is highly expressed in the brain choroid plexus and largely contributes to CSF production.

In a mouse model of hydrocephalus, we demonstrated that the level of functional (phosphorylated) NKCC1 (pNKCC1) is significantly upregulated in the choroid plexus luminal membrane. Here, we tested the potential of gene therapy application by adeno-associated viral vector (AAV) expressing artificial microRNA (amiR) targeting NKCC1 in the hydrocephalus model. Results showed that NKCC1 knockdown significantly rescued the cognitive and motor deficits observed in hydrocephalic mice while histological analysis demonstrated that NKCC1 amiR treatment reduced ventricular enlargement compared to controls. Our data provides evidence that NKCC1 knockdown gene therapy could become a new therapeutic strategy for people with hydrocephalus in the future.

Poster Abstract – Session 1

P13

The role of CHIP in the pathogenesis of repeat expansion diseases

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Repeat expansion diseases (RENDs) are progressive neurodegenerative disorders that include spinal and bulbar muscular atrophy (SBMA) and spinocerebellar ataxia 17 (SCA17). SBMA is caused by CAG expansion in the androgen receptor (AR) gene, whereas SCA17 results from CAG/CAA expansion in the TATA-binding protein (TBP) gene, leading to elongated polyglutamine (polyQ) tracts and nuclear aggregation. TBP alleles with 41–46 repeats show incomplete penetrance. The STUB1 gene, encoding the co-chaperone/E3 ligase CHIP, acts as a disease modifier, giving rise to a digenic form (SCA17-DI). CHIP promotes AR and TBP degradation, reducing aggregation. We demonstrated that CHIP mutants form inclusions and display reduced degradative activity in neuronal cells. To model these mechanisms in a patient-specific context, we generated iPSC lines from fibroblasts of a monogenic SCA17 patient, a healthy donor with an intermediate TBP allele, and a SCA17-DI patient carrying a STUB1 mutation. These lines were differentiated into neuronal precursors by small molecule protocols, providing a platform to study TBP–CHIP interactions and the effects of STUB1 mutations on protein quality control, including genes linked to chaperone-assisted autophagy (HSPA8, HSPB8, BAG3). Finally, we apply molecular approaches to assess how STUB1 mutations affect AR aggregation and degradation, further elucidating CHIP's role as a modifier in RENDs.

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Poster Abstract – Session 1

P14

Early retinal degeneration in amyotrophic lateral sclerosis: preclinical evidence from SOD1G93A mouse model

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease primarily involving motor neuron loss. However, ALS is increasingly recognized as a multisystem disorder affecting other areas of the central nervous system, including the retina. Due to its high susceptibility to metabolic stress, the retina may display early ALS-related alterations before motor symptoms appear. This study investigates retinal changes preceding motor impairment using the transgenic SOD1G93A mouse model, which recapitulates key features of human ALS and develops motor symptoms around postnatal day (PD) 120. Functional, morphological, and molecular analyses were performed at PD55, 70 and 90. Our results revealed early retinal dysfunction starting at PD70, primarily affecting retinal ganglion cells (RGCs). Molecular analyses showed increased levels of oxidative stress and pro-inflammatory markers associated with mutant SOD1 accumulation in the RGC layer. In parallel, miRNome profiling identified four dysregulated miRNAs mainly involved in oxidative and inflammatory pathways. Based on these findings, we selected an adenoviral vector capable of efficiently transducing RGCs, among the most vulnerable retinal neurons in ALS, to develop a potential approach to counteract early ALS-related retinal degeneration. Overall, our results demonstrate early retinal involvement in ALS and support the retina as a target for RNA-based, non-invasive therapeutic strategies against ALS-associated neurodegeneration.

Poster Abstract – Session 1

P15

Mutant Spartin impairs transcription of genes involved in bioenergetics metabolism and alters the mitochondrial protein import

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Pathogenic variants in SPART gene cause Troyer syndrome, a recessive form of spastic paraplegia with lower extremity spasticity and weakness, short stature and cognitive impairment. RNA-sequencing in two cell models of SPART loss-of-function highlighted the downregulation of pathways involved in mitochondrial organization and function, confirmed by bioenergetic studies that revealed profound bioenergetic defects. In both SPART-mutant cell models, we observed an alteration in MAM-associated proteins and an increased distance between the ER and mitochondria, and for the first time we demonstrated that SPART localizes at MAM complexes. In both SPART-mutant cell models, we observed reduced intracellular and endoplasmic reticulum Ca^{2+} levels and mitochondrial protein import which were rescued by overexpression of wild-type SPART. We observed a decreased content of CoQ10. In vitro, CoQ10 supplementation recovered the cell proliferation and ATP production in mutant cells, and in two patients with the homozygous SPART frameshift mutations, six months of CoQ10 treatment reduced fatigability and increased endurance, an encouraging outcome in the context of a neurodegenerative disease. Our findings highlight the critical role of Spartin, as its loss-of-function leads to dysregulation of energy metabolism and mitochondrial organization. Moreover, our data support CoQ10 as a potential therapeutic approach for patients with Troyer syndrome.

Poster Abstract – Session 1

P16

catGRANULE 2.0: accurate predictions of liquid-liquid phase separating proteins at single amino acid resolution

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Liquid-liquid phase separation (LLPS) enables the formation of membraneless organelles, essential for cellular organization and implicated in diseases. We introduce catGRANULE 2.0 ROBOT, an algorithm integrating physicochemical properties and AlphaFold-derived structural features to predict LLPS at single-amino-acid resolution. The method achieves high performance and reliably evaluates mutation effects on LLPS propensity, providing detailed predictions of how specific mutations enhance or inhibit phase separation. Supported by experimental validations, including microscopy data, it predicts LLPS across diverse organisms and cellular compartments, offering valuable insights into LLPS mechanisms and mutational impacts. The tool is freely available at https://tools.tartaglialab.com/catgranule2 and https://doi.org/10.5281/zenodo.14205831.

Poster Abstract – Session 1

P17

Regain ubiquitin-mediated signalling at DNA breaks to promote repair in ALS

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DNA double strand breaks (DSBs) are cytotoxic lesions which activate the DNA Damage Response (DDR) signalling cascade. Key histone mark at DSBs is the ubiquitination H2A o H2AX (Ub-H2A/X). This modification is necessary for DNA repair in post-mitotic cells. Notably, neurons from ALS patients carrying FUSP525L or TDP-43 aggregates exhibit defective DNA repair and accumulation of DNA damage, a hallmark of neuronal stress in ALS. We observed that FUSP525L and TDP-43 aggregates, hinder the recruitment to DSBs of the ubiquitin-ligase RNF168 thus reducing histones ubiquitination. Lack of chromatin ubiquitination at DSBs is known to cause defective DNA repair. Importantly, we discovered that silencing of the specific de-inquinating enzyme (DUB) that counteracts RNF168-dependent ubiquitination at DSB, is sufficient to restore Ub-H2A/X and DNA repair in cells with FUSP525L or TDP-43 aggregates. We are now testing Antisense Oligonucleotide (ASO) to efficiently and specifically silence this DUB in neuronal cells and in FUS linked ALS preclinical mouse models. The identification of these ASOs might represent a potent tool to validate this new pharmacological target in vivo in ALS.

Poster Abstract – Session 1

P18

Exploring the role of the piRNA pathway in microglia and neuroinflammation

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PIWI proteins and the piwi-interacting RNAs (piRNAs) are known in germline stem cells to preserve genome integrity through the epigenetic and post-transcriptional silencing of transposable elements (TEs), repeats and mRNAs. Altered piRNA expression is linked to neural diseases, yet their role in the central nervous system (CNS) remains unclear. We recently found that Piwil2 and piRNAs in neural stem cells of the adult mouse hippocampus support neurogenesis and regulate senescence and neuroinflammation. Here we propose that the piRNA pathway is also present in microglia, the CNS immune cells, and regulate their inflammatory response.

We found that key piRNA pathway genes are expressed in mouse and human microglia, with Piwil2 expression increasing in both acute (lipopolysaccharide (LPS)-induced) or chronic (aging) inflammation. Notably, Piwil2-knockdown in microglia reduces LPS-induced inflammatory cytokines expression and alters mitochondrial metabolism, suggesting a pro-inflammatory role of Piwil2 in these cells. Moreover, neuroinflammation alters the expression of Piwil2-dependent piRNAs, likely representing secondary piRNAs arising from the suppression of transcripts, including TEs and mRNAs.

Our results shed new light on piRNA pathway functions in the CNS, particularly in microglia and neuroinflammation, underpinning the possible involvement of this pathway in neurodegeneration.

Poster Abstract – Session 1

P19

High-Affinity RNA Aptamers Prevent Aberrant Aggregation of FUS

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Fused in sarcoma (FUS) is a multitasking DNA/RNA-binding protein involved in nucleic acid metabolism. Its aggregation into insoluble inclusions is a critical event in the onset and progression of several neurodegenerative diseases. Understanding and preventing its aggregation is paramount for developing potential therapies. Here, we exploit the use of RNA aptamers—short oligonucleotides with defined 3D structures—to interfere with aberrant FUS self-assembly. We hypothesize that specific RNAs can stabilize the protein's native conformation, preventing pathological aggregation. FUS-targeting aptamers were designed using the catRapid algorithm, which predicts protein-RNA interaction strength. Using biolayer interferometry, these aptamers showed unprecedented affinity for FUS. Aggregation assays demonstrated that this interaction fully prevents the formation of aberrant FUS aggregates, keeping the protein in its soluble state. Furthermore, in SK-N-BE neuroblastoma cells expressing either wild-type FUS or the ALS-associated P525L mutant, these aptamers favoured the clearance of toxic insoluble condensates. Treated cells showed significantly improved recovery from stress compared to controls, highlighting their therapeutic potential. This study provides strong evidence that exploiting protein-RNA interactions can prevent protein aggregation. These aptamers represent a rational anti-aggregation strategy to increase protein solubility and could be applied to diverse proteinopathies.

Poster Abstract – Session 1

P20

Morpho-Functional Characterization and miRNA Profiling of the Retina in the 5xFAD Murine Model of Alzheimer's Disease

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Alzheimer's disease (AD) represents the most common neurodegenerative disorder worldwide, characterized by a progressive and irreversible cognitive decline and behavioural defects. The steadily growing prevalence of AD has prompted the scientific community to strengthen its efforts toward the development of innovative diagnostic tools and therapeutic strategies, including genetic interventions such as gene editing and RNA-based therapies. In this context, the retina, as an extension of the central nervous system, is increasingly recognized as a potential window for earlier non-invasive diagnosis of AD. The present work aims to characterize the AD-associated retinal changes in 3-, 6-, and 9-month-old 5xFAD mice. Electroretinography and optical coherence tomography showed reduced retinal activity and deep structural changes in the inner retinal layers of 5xFAD mice starting from 6 months of age. Molecular investigations highlighted high levels of amyloid beta (Aβ) in 5xFAD mice starting from 6 months, mostly localized in the inner retinal layers. An increased expression of hyperphosphorylated Tau and neuroinflammatory, oxidative stress, and apoptotic markers was also found in 5xFAD mice at 9 months. The retinal miRNome of 5xFAD mice revealed the presence of some downregulated miRNAs that are likely to be involved in AD pathogenesis. An adenoviral vector targeting retinal ganglion cells was selected to induce the expression of downregulated miRNAs.

Poster Abstract – Session 1

P21

RNA-based targeting of P2X7R to halt neuroinflammation in Alzheimer's disease

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Growing evidence suggests that neuroinflammation plays a crucial role in Alzheimer's disease (AD) onset and progression. We explored whether and how the extracellular ATP (eATP)/P2X7 receptor (P2X7R) signalling axis influences glia activation, neuroinflammatory pathways and neuronal Ca2+ excitability in the B6.152H AD mouse model. We detected an early rise in brain eATP—prior to Aβ plaque deposition—alongside with inflammasome activation, microglial reactivity and neuronal hyperexcitability. These effects were absent in P2X7R-deficient AD mice, highlighting the key role of P2X7R in setting this early phenotype. We thus designed an RNA-based strategy to silence P2rx7 in AD mice using shRNA delivered via AAV vectors. One month post-intracortical injection, we confirmed transduction via RFP expression and P2X7R downregulation confined to the targeted area, with no evident side effects. Preliminary 2-photon imaging data show that P2rx7 silencing rescues neuronal hyperexcitability in AD mice. Ongoing work is assessing effects on microglia reactivity and neuroinflammation. Similarly, we are setting an alternative approach to downregulate P2X7R in AD mice by intranasal administration of nanoparticles carrying specific siRNA. Our findings suggest that spatially controlled RNA-based silencing of P2rx7 represents a promising and safe therapeutic avenue for modulating early neuroinflammation and neuronal hyperexcitability in AD.

Poster Abstract – Session 1

P22

Retrotransposons-derived regulatory RNAs constrain behavioral flexibility

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To survive, organisms must adapt their behavior in response to environmental changes. While long-term adaptations are critical in evolution, rapid and reversible behavioral adjustments allow organisms to efficiently respond to immediate changes in environmental contingencies, a neuroscience construct defined as behavioral flexibility. On the other ends, in stable environments, repeated behaviors may become rigid and habitual, reducing the need for flexible control. Neural mechanisms underlying both flexibility and rigidity depend on plasticity at corticostriatal circuits.

In this study, we show that behavioral inflexibility is associated with increased RNA levels of Long Interspersed Nuclear Elements -1 (L1) in the lateral part of the dorsolateral striatum (DLS). L1 RNAs sequesters FMRP (Fragile X Messenger Ribonucleoprotein), impairing the availability of key mRNA targets crucial for synaptic processes involved in updating behavioral strategy during changes in contingency. This L1 upregulation is cell-type-specific and affects cortico-striatal postsynaptic strength.

We are currently investigating the contribution of dendritic spine structural plasticity and exploring upstream signals involved in L1 RNA upregulation using large-scale population imaging. These results will further elucidate the role of L1 RNAs in behavioral inflexibility, a phenotypical trait relevant to disorders such as autism-spectrum disorders.

Poster Abstract – Session 1

P23

Self-assembling nanoparticles for a combined RNA-phosphatidylinositol therapy against amyotrophic lateral sclerosis

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Neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS) induce neuronal degeneration which ultimately leads to death. There is currently no cure for ALS [1] and this scenario is further exacerbated by the challenges associated with drug delivery to the brain. We have recently developed a new lipid-based nanoparticle formulation, named self-assembling nanoparticles (SANP) with a calcium phosphate (CaP) core enclosed by a lipid shell able to deliver RNA to the brain [2].

Here, we engineered SANP with a phosphatidylinositol (PIP) derivative, which restores lysosomal calcium homeostasis and autophagy flux, and an antisense microRNA (anti-miRNA) able to prevent the progression of neurodegenerative damage in ALS. SANP formulations were characterized in terms of colloidal properties, RNA and PIP encapsulation, and stability against aggregation in complex fluids. We performed in vitro studies on motoneurons to study the effect of PIP-SANP formulations on calcium efflux from lysosomes and we observed a synergic reduction of the expression of an ALS protein marker when SANP co-encapsulating PIP and anti-miRNA were used. In vivo studies in a mouse model of ALS showed that the PIP-SANP formulations could improve the overall survival of mice, which also retained part of their motor skills. The SANP platform may represent a promising therapeutic approach for the combinatorial treatment of ALS.

[1] doi: 10.1038/nrdp.2017.71.

[2] doi: 10.1016/j.ijpharm.2020."

Poster Abstract – Session 1

P24

AntagomiR-loaded LNPs as a strategy for the treatment of stroke

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Given their ability to regulate the expression of genes involved in the response to stroke, microRNA (miRNA) are key effectors in the pathophysiology of stroke. The 103/107 miRNA family has recently emerged as a therapeutic target in stroke since it controls the expression of a plasma membrane transporter that plays a fundamental role in stroke pathophysiology. However, systemic miRNA delivery requires the use of nanocarriers to prevent degradation by endogenous nucleases and to facilitate intracellular delivery.

Here, we engineered lipid nanoparticles (LNPs) to deliver a mixture of antagomirs (i.e., anti-miRNA103/107) to the central nervous system; we functionalized the LNP surface with transferrin to facilitate crossing of the blood-brain-barrier. The efficacy of the designed LNPs was probed in vitro on primary neuronal cortical cells exposed to hypoxia-mimicking conditions and in vivo on rats exposed to transient occlusion of the middle cerebral artery as a model for stroke. The encapsulation of anti-miRNA103/107 in transferrin-conjugated LNPs allowed blood-brain barrier crossing and significantly reduced brain ischemic damage, paving the way towards a systemic miRNA therapy to tackle stroke.

Poster Abstract – Session 1

P25

Alpha-synuclein autoantibody profiling in Parkinson's Disease patients for pathology prediction

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Parkinson's disease (PD) is a complex neurodegenerative disorder characterized by significant clinical heterogeneity. We investigated a cohort of 100 PD patients, comprehensively characterizing them across more than ten quantifiable variables, including genetic profiles, Unified Parkinson's Disease Rating Scale (UPDRS) scores, and a panel of fluid-based biomarkers. A key focus was the assessment of autoantibodies against alpha-synuclein (aSyn), a protein central to PD pathogenesis. Our initial screening, employing an ELISA-based method, revealed that most PD patients exhibited detectable levels of aSyn autoantibodies, underscoring the prevalence of an autoimmune component in a significant subset of the patient population. To further dissect the specificity of this autoimmune response, we employed a high-throughput microarray-based screening approach, which allowed us for the simultaneous identification of numerous recurring epitopes within aSyn that were recognized by patients' autoantibodies. The rich dataset of identified epitopes was then leveraged as input for a machine learning algorithm to predict the patients' pathological status or progression based on the unique presence and patterns of these specific aSyn autoantibodies. This innovative approach aims to identify novel, non-invasive biomarkers that could aid in the diagnosis, stratification, and potentially prognosis of PD patients, moving towards a more personalized medicine paradigm for this debilitating disease.

Poster Abstract – Session 1

P26

Advancing mRNA Therapeutics with CPP–Nanocomplex Encapsulation in Microparticle-based Microneedles

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Lipid nanoparticles are dominating mRNA delivery but show limitations in terms of endosomal escape, which may reduce gene therapy efficacy. To face this, we developed a hierarchical platform made of PLGA microparticle (MP)-loaded microneedles (MNs) (2) encapsulating mRNA-cell penetrating peptides (CPP) nanocomplexes (NCs). CPPs, already known to facilitate intracellular uptake and provide a potential strategy for the transport of macromolecules through the blood–brain barrier, were investigated for complexation with Cy5-labelled E-GFP mRNA. p5RHH was tested for complexation with Cy5 E-GFP mRNA. NCs were encapsulated into MPs by double emulsion solvent evaporation and characterized by light scattering, confocal and electron microscopy. MPs showed spherical morphology, average size 10 μm, and a porous structure. Agarose gel electrophoresis and fluorescence analysis confirmed mRNA stability. Then we loaded MPs into MNs to deliver NCs, we characterised it morphologically and evaluated the mRNA stability after the fabrication. Then, we tested the mechanical properties and the implantation capability on a skin-like model and studied it with confocal microscopy. The indented model verified the correct implantation of MNs. Our MNs system allows minimally invasive administration and improved stability with the possibility to control kinetics, paving the way for next-generation vaccines. Future steps will assess its functionality in an advanced 3D model.

Poster Abstract – Session 1

P27

Gene Therapy via lipidic Nanomedicines: mRNA and CAS9 delivery

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Gene therapy and nanoparticle (NP)-based drug delivery are revolutionizing therapeutic strategies. The main challenge is developing scalable, non-viral delivery systems tailored to both the pathology and the specific therapeutic payload. In our lab, we optimize microfluidic methods to formulate lipid-based nanoparticles (LNPs) for various applications, showing how precise formulations can serve as versatile platforms.

We designed LNPs for CRISPR/Cas9 delivery to hematopoietic stem cells, targeting neurometabolic disorders. We started with a model enzyme mimicking the ribonucleoprotein (RNP) complex's size and charge. Screening key parameters like lipid composition, flow rate ratio (FRR), and total flow rate (TFR), we identified 16 promising formulations. After enzyme encapsulation, nine LNPs remained monodisperse. The top performer, SCD5, showed 87% uptake in hematopoietic stem cells with a good safety profile. With the RNP complex, we achieved over 50% encapsulation efficiency, confirming its potential for gene editing.

Separately, we've worked on cholesterol (CHOL)-based NPs for Huntington's Disease (HD) treatment. We recently optimized a microfluidic method to maximize CHOL content in NPs while minimizing surfactants, ensuring stability and high brain-targeting efficiency via a scalable protocol. To integrate gene therapy, a cationic lipid was added to allow to laod mRNA and siRNA.

Our studies demonstrate the potential of optimized microfluidic LNPs for gene therapy.

Poster Abstract – Session 2

P28

LNP-mRNA-Based Therapeutics for Genetic Mitochondrial Hepato-Encephalopathies

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Mitochondrial disorders are a heterogeneous group of genetic diseases with a broad clinical involvement caused by mutations in mitochondrial DNA or nuclear genes encoding mitochondrial proteins. Among them, MPV17 mutations are responsible for a severe early-onset hepato-cerebral form of Mitochondrial DNA Depletion Syndromes, characterized by a significant reduction in of mitochondrial DNA copy number in the affected tissues, liver and brain. Current treatments are mostly supportive, with no curative options. The objective of this study is to develop an mRNA-based therapy with the aid of novel lipid nanoparticles targeting mitochondrial disorders, specifically for MPV17-related syndrome in in vitro and in vivo models. Delivery strategies were optimized in hepatic, neuronal, and patient-derived MPV17-mutated cell lines, prompting the possibility of testing mRNA efficacy and LNP targeting. Alongside in vivo experiments demonstrate the potential of lipid-encapsulated mRNA as an effective delivery system with promising results in both liver and brain. Various administration routes and dosages were tested to enhance tissue distribution and expression in the target organs with the aim of shifting to the therapeutic MPV17-mRNA. These findings offer new insight into mRNA-LNP non-viral gene therapy, specifically focusing on its application in addressing multisystemic disorders, holding significant promise for advancing therapeutic strategies for mitochondrial disorders.

Poster Abstract – Session 2

P29

Unbiased identification of microRNAs with a protective action on inherited photoreceptor degeneration through in vitro high-content screening

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Inherited retinal diseases (IRDs) are clinically and genetically heterogeneous disorders characterized by progressive photoreceptor degeneration and irreversible vision loss. MicroRNAs (miRNAs), a class of endogenous non-coding RNAs with regulatory properties, are known to play a major role in retinal function. Given their ability to simultaneously modulate multiple molecular pathways, miRNAs represent promising therapeutic tools for genetically heterogeneous disorders, such as IRDs. In this study, we performed a high-content imaging (HCI) in vitro screening to systematically assess the impact of miRNA overexpression on photoreceptors. Over 1,200 miRNAs were assayed for putative protective or detrimental effects in 661W photoreceptor-like cells undergoing light-induced degeneration. Potentially cell-protective miRNAs highlighted by this screen were first validated in vitro and then tested in vivo through adeno-associated viral (AAV) vector retinal administration to an IRD mouse model. In vivo effects were analysed by functional and morphological assays and, in selected cases, RNAseq approaches. miR-429 displayed the strongest cell-protective effect in vitro. Subretinal delivery of miR-429 in the RhoP23H/+ mouse model preserved electrophysiological responses and was associated with reduced inflammatory processes in the retina. Modulation of nine additional miRNAs identified through the HCI screen is currently being evaluated in vivo. We demonstrate that the HCI assay we devise

Poster Abstract – Session 2

P30

FBXO34: a potential therapeutic target for Huntington's disease

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Huntington's disease (HD) is a neurodegenerative disorder that affects striatal neurons. It is caused by an expansion of CAG repeats (>35) in the Huntingtin gene, resulting in the production of the mutant HTT (mHTT) protein. This aberrant protein disrupts the processes within cells, causing toxicity and cell death. Despite progress in understanding the causes of HD, there are still no effective treatments. To address this challenge, our research group conducted an unbiased genome-wide screening to identify potential target genes capable to mitigate mHTT toxicity. This approach led to the identification of over 100 candidate genes. The main focus of this research is FBXO34, a poorly understood gene belonging to the F-box protein family that plays an essential role in the protein ubiquitination process. First, we validated the protective effects of Fbxo34 using in vitro and in vivo models of HD, including mouse embryonic stem cells (mESCs), zebrafish, and R6/2 mice. In each model, Fbxo34 rescued mHTT phenotypes. We are now focused on understanding the biological effect of Fbxo34 in the context of HD pathogenesis. We leveraged mESCs to verify the involvement of Fbxo34 in the ubiquitination process and in mHTT degradation. Our next steps will include the validation of the Fbxo34 role in vitro human models derived from iPSCs: medium spiny neurons and striatal organoids. The final aim is to develop RNA-based delivery strategies, using FBXO34-mRNA as potential therapy against HD.

Poster Abstract – Session 2

P31

Understanding SPG9: a rare form of Hereditary Spastic Paraplegia

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Hereditary Spastic Paraplegia type 9 (SPG9) is a rare genetic neurodegenerative disorder caused by mutations in the ALDH18A1 gene.

This study aims to provide a deeper understanding of the pathogenetic mechanism of SPG9 and therapeutic options.

We approached this project by 1) deriving a cellular model based on a patient-derived fibroblast cell line carrying an ALDH18A1 pathogenic variant (NM_002860.4: c.727G>C, p.Val243Leu) and 2) generating a mouse model bearing the same mutation in the mouse Aldh18a1 gene. Furthermore, we designed editing tools to revert the p.Val243Leu mutation found in patients.

Our analyses of the SPG9 cell model revealed distinct phenotypes that are the results of alterations in cell dimensions, growth pattern, and mitochondrial features such as ROS production, ATP synthesis, by phalloidin staining, MTT analysis, galactose growth compared to WT cells. The transgenic mouse showed a movement disorder phenotype, strengthening its value as SPG9 model.

Furthermore, we explored amino acid supplementation as a novel therapeutic approach in our in vitro model.

As preliminary results we identified specific phenotypes in our models, and that specific amino acid supplementation may hold promise as a potential treatment strategy for SPG9, offering a targeted approach to mitigate cellular dysfunction associated with ALDH18A1 gene mutations.

Poster Abstract – Session 2

P32

Small Non-Coding RNAs Dysregulation in the Microglia from a Mouse Model of Down Syndrome

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Microglia are the brain's resident immune cells and regulate a wide range of mechanisms critical for neuronal activity and cognitive functions. Down Syndrome (DS), is a genetic disorder caused by trisomy of human chromosome 21 (Hsa21), characterized by intellectual disability and multiple health issues, including immune system alterations. In both individuals with DS and DS mouse models, such as Dp(16), microglia overactivation has been observed, correlating with impaired neuronal function and cognitive deficits.

Microglial inflammatory response is tightly regulated by small non-coding RNAs, potentially representing novel targets for therapeutic interventions.

In this study, we investigated small non coding RNAs such as microRNAs (miRNAs) and PIWI-interacting RNAs (piRNAs) in microglia isolated from Dp(16) mice. Several of the miRNAs that we found upregulated in DS microglia were also upregulated in mouse models of acute inflammation, supporting a sustained inflammatory microglial state in DS, which may affect neuronal function. Additionally, we observed increased expression of Piwil2 (Mili), a key effector of piRNA biogenesis and function, together with altered piRNAs expression profile, suggesting a dysregulated piRNA pathway in DS microglia.

Our findings indicate that small non-coding RNA dysregulation may drive microglial dysfunction in DS, identifying these pathways as potential diagnostic molecules and therapeutic targets.

Poster Abstract – Session 2

P33

Microfluidic bioprinting of a human neurovascular model for CNS drug screening and Kabuki Syndrome modeling

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Three-dimensional (3D) neural culture models better replicate the in vivo microenvironment than traditional 2D systems, supporting enhanced neuronal integration, maturation, and synaptogenesis. However, most 3D models lack vascularization, limiting physiological relevance given the importance of angiogenesis in neural development. This study introduces a novel 3D bioprinting strategy to create vascularized neural tissue using iPSC-derived neural precursors and human endothelial cells. A custom microfluidic printhead generates core-shell fibers with a soft neural core and a stiffer endothelial shell, mimicking the cellular architecture of the human blood-brain barrier (BBB). The constructs are designed for bioreactor perfusion to apply physiological shear stress, encouraging endothelial alignment and prolonged neural viability. This platform offers a promising tool for CNS drug screening and disease modeling. As a case study, we apply this system to Kabuki Syndrome type 1 (KS1), a neurodevelopmental disorder linked to KMT2D mutations. While KMT2D is known to regulate enhancer function and neuronal differentiation, its impact on human neurodevelopment remains unclear. Our model enables mechanistic studies of KS1 and therapeutic testing in a human-relevant 3D neural-vascular environment.

Poster Abstract – Session 2

P34

Multi-SINEUP: a novel RNA therapeutic approach for 22q11.2 microdeletion syndrome

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SINEUPs are a class of natural and synthetic antisense long non-coding RNAs with the function of enhancing translation of sense mRNAs. Their activity is mediated by two key regions: the binding domain (BD), which provides target specificity through sequence complementarity, and the effector domain (ED), an inverted SINEB2 element that drives translational upregulation. By engineering artificial BDs, synthetic SINEUPs can be tailored to boost the protein output of virtually any gene. Previous studies have demonstrated their efficacy in both cell-based systems and animal models, confirming their broad potential as promising therapeutic tools for haploinsufficiency disorders, which result in insufficient protein production. In chromosomal microdeletions, multiple genes are simultaneously affected. One example is 22q11.2 deletion syndrome, a complex condition with multi-organ involvements. Current therapies typically address single genes, leaving multi-gene syndromes with limited treatment options. In this study, we designed the first multi-BD-SINEUP to target three genes—TBX1, COMT, and DGCR8—within the 22q11.2 region. We showed that this construct successfully increased protein levels in vitro in cells and in vivo in mouse brain. Importantly, it also rescued cognitive impairments in LgDel mice, a model of the syndrome. Thus, we provide proof-of-concept for multi-BD-SINEUPs as a novel strategy to restore expression of multiple haploinsufficient genes simultaneously.

Poster Abstract – Session 2

P35

SINEUP-RIT2 as therapeutic strategy in Parkinson´s Disease

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"Parkinson's Disease (PD) is a neurodegenerative disorder characterized by loss of Substantia Nigra dopamine neurons and accumulation of phosphorylated α-Synuclein (pS129-αSyn) in surviving ones. The major unmet clinical need in PD is the lack of disease-modifying treatments.

The genetic locus of the small GTPase RIT2 is associated with PD, and RIT2 mRNA is downregulated in idiopathic PD patient's brains and PD models. Increasing RIT2 in PD models overexpressing G2019S-LRRK2 or A53T-αSyn reduces LRRK2 activity, αSyn pathology, lysosomal dysfunction, and neurodegeneration, indicating therapeutic potential.

We developed SINEUP (Short Interspersed Nuclear Element B2 UP regulation) molecules targeting RIT2, to enhance its endogenous translation, avoiding overexpression- and ectopic-related toxicity.

We designed five SINEUP-RIT2 molecules and delivered them into murine Neuro2A cells identifying two that increase RIT2 protein without affecting the mRNA. SINEUP-dependent elevation of RIT2 correlated with increased p38-MAPK phosphorylation, a downstream effector of RIT2, suggesting functional effects of SINEUP-driven translation.

We are testing the two most effective SINEUP-RIT2 molecules in LRRK2-G2019S SH-SY5Y cells, which model key cellular LRRK2-PD features. Preliminary results show reduced pS129-αSyn inclusions and LRRK2 activity, mirroring RIT2 overexpression. These findings support SINEUP-mediated RIT2 upregulation as a potential neuroprotective strategy for PD."

Poster Abstract – Session 2

P36

The Pgbd5 DNA transposase controls DNA double-strand breaks formation during cerebral cortex development

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PiggyBac domain (PGBD) transposons are highly conserved elements present across a wide range of organisms, from fungi to humans. In unicellular eukaryotes, homologs of these transposons are involved in DNA-programmed elimination. In vertebrates, the PiggyBac Transposable Element Derived 5 (Pgbd5) gene was domesticated more than 500 million years ago. Pgbd5 is predominantly expressed in the central nervous system, retains endonuclease activity, yet its physiological function remains largely unknown.

In this study, we demonstrate that targeted in vivo manipulation of Pgbd5 expression significantly alters neural cell type composition and impair their migratory behavior during mouse corticogenesis. These effects are accompanied by a marked reduction in the formation of endogenous DNA double-strand breaks (DSBs) in neural cells, without evidence of increased cell death or somatic genomic rearrangements.

Collectively, our findings identify Pgbd5 as a transposase-derived protein that plays a critical role in establishing the molecular environment necessary for proper mammalian brain development.

Poster Abstract – Session 2

P37

SINEUP RNAs: a new platform for treating haploinsufficiency in Autism Spectrum Disorders (ASD)

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Autism Spectrum Disorder (ASD) is a highly heterogeneous condition and most of the currently known high-correlated risk genes harbor de novo disruptive variants, causing haploinsufficiency. A potential therapeutic avenue resides in stimulating the expression from the not affected allele to restore protein to physiological levels. Our project focuses on the use of SINEUPs, a functional class of antisense lncRNAs able to increase target protein levels by promoting the translation of partially overlapping sense mRNAs. Because of their modular structure, they can be artificially engineered to increase the production of virtually any gene of interest. In recent investigations of the laboratory, the chromodomain helicase DNA-binding 8 (CHD8), has been targeted successfully. We employed synthetic SINEUP-CHD8 to efficiently stimulate endogenous CHD8 protein production. Indeed, SINEUP-CHD8 were effective in human cells with reduced levels of the target protein and in patients'-derived fibroblasts to revert molecular phenotypes associated with CHD8-suppression. In this project, we proposed to translate the knowledge gained by studying SINEUP-CHD8 in vitro to discover new SINEUPs targeting other ASD risk factors causing dominant haploinsufficiencies. Specifically, we aimed to target 7 ASD-risk candidate genes [ADNP, CHD2, DYRK1A, GRIN2B, SCN2A, STXBP1, SYNGAP1] and test whether SINEUP directed against these targets might rescue protein's levels and functionality in ASD.

Poster Abstract – Session 2

P38

Strategies to promote the expression of the androgen receptor AR-A isoform lacking the polyglutamine tract in spinal and bulbar muscular atrophy

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Spinal and bulbar muscular atrophy is a neuromuscular disorder caused by a toxic polyglutamine (polyQ) expansion in the androgen receptor (AR). An isoform of the AR (AR-A) results from translation driven by an AUG(-II) downstream to the canonical AUG-I of AR. AR-A isoform lacks part of the N-terminal domain, including the polyQ tract. Redirecting the translation start site to the AUG-II might represent a strategy to avoid the expression of the toxic ARpolyQ, while preserving partial AR function of the shorter AR-A isoform. Strategies to switch the translation to AR-A isoform include the use of antisense oligonucleotides (ASO) to induce a steric blockage at the AUG-I encoding AR-B. To screen the ASO ability to switch AR-B to AR-A translation, we generated a reporter SH-SY5Y cell line (pScreen) expressing GFP or HiBit in frame with AUG-I or AUG-II, respectively. Ten ASOs were tested on pScreen cells, followed by HiBiT assay and GFP detection through western blot. The HiBiT assay showed that one ASO (#6) increased HiBiT-related luminescence normalised to cell viability, suggesting the induction of translation starting from the AUG-II. Instead, when we evaluated the reporter for AUG-I translation, ASO #6 also decreased GFP protein levels. In summary, there is evidence that the expression of AR isoforms can be modulated; however, the mechanisms underlying this regulation require further validation. Fundings: CN RNA & Gene Therapy, PNRR, CN3, Project CN00000041, Spoke 3

Poster Abstract – Session 2

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Gene Therapy in Huntington's Disease: Functional Validation Through Early Phenotype Rescue and Multi-Omics Profiling

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Huntington's disease (HD) is an inherited and incurable neurodegenerative disorder caused by CAG repeat expansions in the huntingtin (HTT) gene. The resulting mutant HTT protein alters cellular physiology at the multiple levels, inducing toxicity and cell death. The mechanisms directly causing toxicity are partially understood, thus precluding the development of successful therapeutic strategies.

In order to implement an effective gene therapy, an unbiased genome-wide screening was performed to detect genes functionally involved in cytotoxicity induced by mutant HTT. This study enabled the identification of potential suppressors of mutant huntingtin, such as Mtf1, which were subsequently tested in vitro, in mouse ES cells, and in vivo, in zebrafish and mouse.

Currently, we are developing human in vitro models, including 2D striatal neurons and 3D organoids, to study HD pathology. However, alongside adult models, recent studies have reported defects also during early development, including impaired polarity, differentiation, and ciliogenesis. We therefore decided to investigate as well eearly phenotypes using neural progenitor cells obtained by differentiating iPSCs bearing 21 or 109 CAG repeats, and rescuing effect of our candidate gene was assessed by analyzing ROS levels pre- and post-delivery, but also through proteomics and transcriptomics analysis.

Poster Abstract – Session 2

P40

Toward Rational Rna Drug Design: Thermodynamic Integration And Alchemical Transfer Methods For Binding Predictions

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RNA is emerging as a promising therapeutic target due to its central role in regulating diverse cellular functions. However, accurately predicting ligand binding affinities to RNA remains a major computational challenge, largely due to RNA's intrinsic flexibility and structural heterogeneity. In this work, we benchmark two alchemical free energy methods—Thermodynamic Integration (TI)1 and the Alchemical Transfer Method (ATM)2—to estimate binding free energies for a representative set of RNA–ligand. By systematically comparing these approaches, we assess their accuracy, robustness, and sensitivity to structural fluctuations. Our findings offer practical guidelines for modelling RNA–ligand interactions and contribute to the development of more reliable tools for RNA-targeted drug design. Ultimately, this work supports the rational design of novel, high-affinity ligands for RNA, advancing the discovery of next-generation RNA-based therapeutics.

Poster Abstract – Session 2

P41

The long non-coding antisense RNA AQP4-AS as a novel regulator of Aquaporin- 4 expression

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LncRNAs are key regulators of gene expression in brain development, aging, and disorders such as AD, PD, autism, and tumors. This study investigates whether AQP4 expression during mouse brain development and aging is regulated by antisense transcripts GM50048-201 and -202 (collectively, AQP4-AS). We analyzed AQP4 mRNA, AQP4-AS, and protein levels from embryonic to aged stages (E18–32M, n=4). At E18–P0, AQP4 mRNA and AQP4-AS levels were low (5–10% of 3M levels), with undetectable protein. AQP4 protein appeared around P3, increasing to a plateau by 1–3M. AQP4-AS consistently exceeded AQP4 mRNA, suggesting a stabilizing, not inhibitory, role. In aged brains (13–32M), AQP4 mRNA, AQP4-AS, and protein levels declined, returning to early developmental values by 32M. We also quantified AQP4 and AQP4-AS levels in human samples, particularly in healthy (HC) and tumor tissues from the same individuals affected by glioblastoma multiforme (GBM) (n=7). Our experiments showed an upregulation of AQP4-AS in GBM compared to HC. This increase in AS transcripts was accompanied by different AQP4 mRNA levels that resulted in both up-regulated (n=3) and downregulated (n=3), coupled with a general decrease in protein levels. RNAi experiments in mouse astrocytes showed that silencing AQP4 reduced AQP4-AS, and silencing AQP4-AS led to a decrease in both AQP4 mRNA and protein. Thus, AQP4-AS appears to positively modulate AQP4 expression. In aging, AQP4-AS helps maintain AQP4 levels, while its dysregula

Poster Abstract – Session 2

P42

Genome-wide analysis of tandem repeat expansions in Autism Spectrum Disorder

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Tandem repeat expansions (TREs) are associated with over 60 monogenic disorders. Recent whole genome sequencing (WGS) studies highlighted their involvement in autism spectrum disorder (ASD), but further studies are necessary to better elucidate the role of specific TREs in ASD risk.

We analyzed WGS data of 105 ASD families using ExpansionHunter Denovo, to identify TREs whose lengths were outliers compared with other members of the cohort. TREs were filtered according to Z-score>10 and TR length>150 bp (high confidence,HC-TREs). Then we used ExpansionHunter to accurately genotype the most interesting TREs, and REViewer to generate read visualizations for all individuals at each locus. The frequency of specific TREs was assessed using TR-gnomAD data. Experimental validation was performed using fluorescent Long-Range PCR (LR-PCR) and Repeat-primed PCR.

We identified 280 HC-TREs from 124 ASD individuals. We then prioritized 10 TRE in genes already implicated in ASD or other neurodevelopmental disorders. Among these, the most interesting one is a rare AAG expansion in the intron 11 of CACNB1, a gene recently highlighted among the top candidate ASD-relevant tandem repeat loci. Interestingly, LR-PCR confirmed that the tandem repeat, expanded in the unaffected mother, is further expanded in the proband.

The identification of tandem repeat expansions in CACNB1 and other ASD/NDD risk genes strengthens the role of rare TRE in the genetic etiology and phenotypic complexity of ASD.

Poster Abstract – Session 2

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circHTT(2,3,4,5,6) and possible implications in Huntington's disease

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"Background: Huntington's disease (HD) is a neurodegenerative disorder caused by a CAG repeat expansion in the HTT gene. We identified circHTT(2,3,4,5,6), a circular RNA from the HTT locus, enriched in neurons, conserved in mammals, and whose expression correlates with CAG length in HD models. Its overexpression alters cell morphology and adhesion, suggesting a role in disease modulation. Sequence analysis predicts RBP binding sites and an IRES; consistent with this, circHTT co-sediments with the 40S ribosomal subunit, implying translational involvement.(Morandell et al., 2024).

Aim: Investigate the cellular and molecular function of circHTT(2,3,4,5,6), its impact on morphology, transcription, translation, and protein interactions.

Methods–Results: We use two stable models: MN9D dopaminergic cells (circHTT KD via Ago-shRNA) and STHdh striatal cells (circHTT OE). Cell painting is ongoing to assess morphology/adhesion upon KD. Translational efficiency analysis revealed changes in gene expression and translation. An ASO-RNA pulldown protocol is being optimized to isolate circHTT and define its interactome via proteomics.

Conclusions: Preliminary data show transcriptional and translational differences upon circHTT KD, involving genes linked to translation. Future work will correlate these findings with phenotypic changes and define circHTT interactors to uncover novel HD mechanisms and therapeutic avenues."

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